SUPPORTING INFORMATION FOR ONLINE PUBLICATION

Fortes et al. Ancient DNA reveals differences in behaviour and sociality between brown bears and extinct cave bears.

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Section 1 – Details of additional experiments not reported in the main text

Novel DNA sequences reported in this study were generated over four separate experiments involving a variety of methods.

Experiment 1

All but one novel Spanish sequence was generated in Experiment 1, details of which are reported in the main text

Experiment 2

The mitogenome sequence from a single Romanian cave bear specimen (PA1) was generated in a separate experiment using the methodology described above, but without initial screening using Sanger sequencing. Sequencing and data processing were as described for Experiment 1.

Experiment 3

Mitogenome sequences of four Austrian cave bears (GS136, GS710, RK306, SW1867) were generated from petrous bone samples that had sufficiently high endogenous DNA content to permit recovery of the mitogenome using shotgun sequencing. Libraries were generated using the double-stranded protocol of Meyer & Kircher (2010) from two DNA extractions obtained from each sample, one using the protocol described by Rohland *et al*. (2010) and the second using an optimised protocol for the recovery of short DNA fragments (Dabney *et al*. 2013). Library amplification and indexing involved a total of 20 cycles of PCR. Indexed libraries were quantified using Agilent Bioanalyzer 2100 and ThermoFisher Scientic Qubit 1.0 instruments, prior to 100 bp paired-end sequencing on the Illumina HiSeq Platform. The program CutAdapt (Martin 2011) was used to trim adapter sequences and remove any trimmed reads < 25 bp. Overlapping read-pairs were then merged using the progam Flash (Magoč & Salzberg 2011). Only merged read-pairs – which comprised the vast majority of read-pairs (Tab. S4) – were used for mapping to the *U. spelaeus* reference mitochondrial genome sequence (GenBank Accession EU327344) in the case of *U. spelaeus* clade individuals, and a *U. ingressus* mitochondrial genome sequence (GenBank Accession NC011112) in the case of *U. ingressus* clade individuals. Consensus sequences were generated with samtools Mpileup (Li & Durbin 2009) and visually compared with the read alignment and corrected where necessary.

Experiment 4

Mitogenome sequences of a Spanish cave bear (E-VD-1838), an Austrian cave bear (WK01) and an Austrian Late Pleistocene brown bear (Uap) were generated from petrous bone samples using shotgun sequencing. DNA extraction followed the protocol of Dabney *et al*. (2013) and libraries were constructed using the single-stranded protocol in Gansauge & Meyer (2013). The optimal number of PCR cycles was estimated using qPCR prior to library indexing, as described in Gansauge and Meyer (2013). Indexed libraries were quantified using Agilent TapeStation 4200 and ThermoFisher Scientic Qubit 2.0 instruments, prior to 75 bp paired-end sequencing on the Illumina NextSeq 500 Sequencing Platform. Data processing followed that described for Experiment 3 except that reads from sample Uap were mapped to the brown bear reference mitochondrial sequence (GenBank Accession EU497665).

Section 2 – PartitionFinder analysis

Optimal partitions and substitution models for the cave bear and brown bear alignments were selected using PartitionFinder (Lanfear et al. 2012) under the Bayesian Information Criterion (BIC) using the greedy search algorithm and linked branch lengths. Substitution models were selected from all models available in the software BEAUTi, that is used to create the input XML file for BEAST.

The optimal partitioning scheme for the cave bear alignment was selected from the following data blocks, which comprise all tRNAs, all ribosomal RNA genes, and individual codon positions of each protein coding gene:

Leu1 = 334 – 412; ND1_CP1 = 413 – 1369\3; ND1_CP2 = 414 – 1369\3; ND1_CP3 = 415 – 1369\3; Ile = 1370 – 1437; Gln = 1438 – 1508; Met = 1509 – 1579; ND2_CP1 = 1580 – 2623\3; ND2_CP2 = 1581 – 2623\3; ND2_CP3 = 1582 – 2623\3; Trp = 2624 – 2690; Ala = 2691 – 2767; Asn = 2768 – 2840; Cys = 2841 – 2946; Tyr = 2947 – 3016; COX1_CP1 = 3017 – 4561\3; COX1_CP2 = 3018 – 4561\3; COX1_CP3 = 3019 – 4561\3; Ser1 = 4562 – 4632; Asp = 4633 – 4703; COX2_CP1 = 4704 – 5387\3; COX2_CP2 = 4705 – 5387\3; COX2_CP3 = 4706 – 5387\3; Lys = 5388 – 5462; ATP8_ATP6_CP1 = 5463 – 6303\3; ATP8_ATP6_CP2 = 5464 - 6303\3; ATP8_ATP6_CP3 = 5465 – 6303\3; COX3_CP1 = 6304 – 7087\3; COX3_CP2 = 6305 – 7087\3; COX3_CP3 = 6306 – 7087\3; Gly = 7088 – 7156; ND3_CP1 = 7157 – 7502\3; ND3_CP2 = 7158 – 7502\3; ND3_CP3 = 7159 – 7502\3; Arg = 7503 – 7573; ND4L_CP1 = 7574 – 7869\3; ND4L_CP2 = 7575 – 7869\3; ND4L_CP3 = 7576 – 7869\3; ND4_CP1 = 7870 – 9247\3; ND4_CP2 = 7871 – 9247\3; ND4_CP3 = 7872 – 9247\3; His = 9248 – 9317; Ser2 = 9318 – 9377; Leu2 = 9378 – 9447; ND5_CP1 = 9448 – 11251\3; ND5_CP2 = 9449 – 11251\3; ND5_CP3 = 9450 – 11251\3; ND6_CP3 = 11252 – 11779\3; ND6_CP2 = 11253 – 11779\3; ND6_CP1 = 11254 – 11779\3; Glu = 11780 – 11852; CYTB_CP1 = 11853 – 12992\3; CYTB_CP2 = 11854 – 12992\3; CYTB_CP3 = 11855 – 12992\3; Thr = 12993 – 13064; Pro = 13065 – 13131; D-loop = 13132 – 14074; Phe = 14075 – 14144; 12s = 14145 – 15133; Val = 15134 – 15221; 16s_p1 = 15222 – 16468; 16s_pt2 = 1 – 333.

For the brown bear alignment, this set of test partitions contained insufficient variability for PartitionFinder analysis, and so each protein-coding gene was treated as a single data block, rather than being split into individual codon positions. For the cave bear alignment, PartitionFinder found best support for a five-partition scheme (Table S5), and for the brown bear alignment, PartitionFinder found best support for a three-partition scheme (Table S6).

Table S1. Geographical location and number of specimens found in each of the Spanish paleontological sites included in this study.

Cave	**Num. in Fig. S1**	**Locality**	**x (W)**	**y (N)**	**Z *(m a.s.l.)**	**Taxon**	**†NISP**	**‡MNI**	**Refs.**
Eirós	1	Triacastela	7º 12’ 21’’	42º 45’ 58’’	780	*U. spelaeus*	≈3000	46	1, 2
Liñares	2	Pedrafita do Cebreiro	7º 04’ 19’’	42º 40’ 58’’	1115	*U. spelaeus*	≈500	15	3
A Ceza	3	Folgoso do Courel	7º 08’ 15’’	42º 40’ 52’’	1005	*U. spelaeus*	≈860	6	4
Arcoia	4	Folgoso do Courel	7º 05’ 06’’	42º 36’ 44’’	1170	*U. spelaeus*	5	3	Unpubl
Rebolal	5	Cobas	6º 48’ 54’’	42º 28’ 08’’	560	*U. spelaeus*	≈150	5	5
Tito Bustillo	6	Ribadesella, molecular	5º 04’ 04’’	43º 27’ 39’’	<5	*U. spelaeus*	≈1000	33	6, 7
Reguerillo	7	Patones	3º 26’ 43’’	40º 53’ 07’’	800	*U. spelaeus*	≈2000	50	8
Troskaeta	8	Ataún	2º 09’ 20’’	43º 00’ 40’’	580	*U. spelaeus*	≈1700	29	9
Amutxate	9	Lekunberri,	1º 58’ 01’’	42º 58’ 25’’	950	*U. spelaeus*	≈15,000	85	10
Purruñal	10	Pedrafita do Cebreiro	7º 05’ 29’’	42º 42’ 29’’	1150	*U. arctos*	42	1	11, 12, 13
Longo de Meu	11	Folgoso do Courel	7º 06’ 58’’	42º 37’ 14’’	775	*U. arctos*	1	1	12
Pena Paleira	12	Folgoso do Courel	7º 07’ 09’’	42º 36’ 57’’	1200	*U. arctos*	196	7	11, 13
Tarelo	13	Folgoso do Courel	7º 07’ 46’’	42º 37’ 39’’	680	*U. arctos*	93	3	11, 13
Sima de los Osos de Somiedo	14	Somiedo	6º 07’ 20’’	43º 03’ 00’’	1900	*U. arctos*	958	9	6, 12, 14
Pozu La Cigacha	15	Teverga	5º 57' 27"	43º 03' 06"	1770	*U. arctos*	41	2	12
Pozu La Veiga'l Retuertu	16	Quirós	5º 56' 71"	43º 03' 18"	1590	*U. arctos*	10	2	12
La Canal Fuerte	17	Campocaso	5º 19’ 48’’	43º 05’ 59’’	1550	*U. arctos*	127	3	12
Red de Toneyo	18	Amieva	5º 02’ 52’’	43º 11’ 22’’	1387	*U. arctos*	9	2	12
Cuetu Blanco	19	Llanes	4º 43’ 53’’	43º 22’ 44’’	655	*U. arctos*	43	1	12

***m.a.s.l.: meters above sea level. †NISP: number of identified specimens. ‡MNI: minimum number of individuals.**

[1]Grandal (1993); [2]Grandal and Vidal (1997); [3]López-González 2003; [4]Grandal and López-González 1998; [5]Grandal *et al* 2006; [6]Pinto Llona *et al* 2005; [7]Pinto Llona *et al* 2006; [8]Torres 1984; [9]Torres *et al* 1991; [10]Torres *et al* 2007; [11]García-Vázquez *et al* 2011; [12]García-Vázquez *et al* 2015; [13]Torres 1983; [14]Pinto Llona 1999.

Table S2. Samples investigated in this study. Symbols + and – indicates the success or failure of PCR test of DNA preservation. Experiment refers to those described in Section 1

Sample ID	**DNA ID**	**Taxon**	**Experiment**	**Locality**	**Material**	**PCR amplification**
E-TD-2503	E1	*U. spelaeus*	1	Eirós	Rib	+
E-V-S-55	E2	*U. spelaeus*	1	Eirós	Rib	+
E-V-S-162	E3	*U. spelaeus*	1	Eirós	Rib	+
E-Ua-S-2623	E4	*U. spelaeus*	1	Eirós	Rib	+
E-ZYX-S-963	E5	*U. spelaeus*	1	Eirós	Radius	+
E-ZYX-S-958	E6	*U. spelaeus*	1	Eirós	Radius	+
E-ZYX-S-964	E7	*U. spelaeus*	1	Eirós	Radius	+
E-ZYX-S-962	E8	*U. spelaeus*	1	Eirós	Radius	+
E-T-3010	E9	*U. spelaeus*	1	Eirós	Rib	+
E-T-3011	E10	*U. spelaeus*	1	Eirós	Rib	+
E-T-3012	E11	*U. spelaeus*	1	Eirós	Rib	+
E-T-3017	E12	*U. spelaeus*	1	Eirós	Rib	+
2112	E13	*U. spelaeus*	1	Eirós	Femur	+
2107	E14	*U. spelaeus*	1	Eirós	Femur	+
2104	E15	*U. spelaeus*	1	Eirós	Radio	+
2544	E16	*U. spelaeus*	1	Eirós	Tibia	+
2583	E17	*U. spelaeus*	1	Eirós	Ulna	+
EUSA-11	E18	*U. spelaeus*	1	Eirós	Rib	-
VL-L-410-G	L1	*U. spelaeus*	1	Liñares	Rib	+
VL-L-406-G	L2	*U. spelaeus*	1	Liñares	Rib	+
VL-1331-G	L3	*U. spelaeus*	1	Liñares	Rib	+
Lin-E230	L4	*U. spelaeus*	1	Liñares	Rib	+
Lin-E144	L5	*U. spelaeus*	1	Liñares	Rib	+
Lin-E227	L6	*U. spelaeus*	1	Liñares	Rib	+
VL-571-G	L7	*U. spelaeus*	1	Liñares	Rib	+
Lin-59	L8	*U. spelaeus*	1	Liñares	Rib	+
VL-L-411-G	L9	*U. spelaeus*	1	Liñares	Rib	+
VL-L-417-G	L10	*U. spelaeus*	1	Liñares	Rib	+
Lin-E-149	L11	*U. spelaeus*	1	Liñares	Rib	+
VL-L-398-G	L12	*U. spelaeus*	1	Liñares	Rib	+
VL-L-405-G	L13	*U. spelaeus*	1	Liñares	Rib	+
Lin-234	L14	*U. spelaeus*	1	Liñares	Rib	+
Lin-E229	L15	*U. spelaeus*	1	Liñares	Rib	+
Lin-549	L16	*U. spelaeus*	1	Liñares	Rib	+
Lin-1009	L17	*U. spelaeus*	1	Liñares	Tibia	+
Lin-E57	L18	*U. spelaeus*	1	Liñares	Rib	+
Lin-1007	L19	*U. spelaeus*	1	Liñares	Rib	+
CEZ-540	C1	*U. spelaeus*	1	A Ceza	Rib	+
CEZ-535	C2	*U. spelaeus*	1	A Ceza	Rib	+
CEZ-534	C3	*U. spelaeus*	1	A Ceza	Rib	+
CEZ-S-276	C4	*U. spelaeus*	1	A Ceza	Rib	+
CEZ-271	C5	*U. spelaeus*	1	A Ceza	Rib	-
CEZ-150	C6	*U. spelaeus*	1	A Ceza	Jaw	-
CEZ-561	C7	*U. spelaeus*	1	A Ceza	Rib	+
CEZ-563	C8	*U. spelaeus*	1	A Ceza	Rib	+
CEZ-565	C9	*U. spelaeus*	1	A Ceza	Rib	+
CEZ-605	C10	*U. spelaeus*	1	A Ceza	Rib	+
CEZ-710	C11	*U. spelaeus*	1	A Ceza	Rib	+
Ar-US-peq	A1	*U. spelaeus*	1	Arcoia	Femur	+
Ar-US-gde	A2	*U. spelaeus*	1	Arcoia	Femur	+
PZ-1	CB-R1	*U. spelaeus*	1	Rebolal	Tooth	-
PZ-15	CB-R2	*U. spelaeus*	1	Rebolal	Humerus	-
PZ-16	CB-R3	*U. spelaeus*	1	Rebolal	Femur	-
PR-R-97-5	CB-R4	*U. spelaeus*	1	Rebolal	Jaw	-

PR-R97-267	CB-R5	*U. spelaeus*	1	Rebolal	Jaw	-
PR-R99-158	CB-R6	*U. spelaeus*	1	Rebolal	Rib	-
TB-99-AA8-002	CB-TB1	*U. spelaeus*	1	Tito Bustillo	Rib	-
TB-99-B2-034	CB-TB2	*U. spelaeus*	1	Tito Bustillo	Rib	-
TB-99-ZB49-050	CB-TB3	*U. spelaeus*	1	Tito Bustillo	Rib	-
TB-2002-C9-008	CB-TB4	*U. spelaeus*	1	Tito Bustillo	Rib	-
T-125	CB-CR1	*U. spelaeus*	1	Reguerillo	Tooth	-
T-1069	CB-CR2	*U. spelaeus*	1	Reguerillo	Tooth	-
T-2325	CB-CR3	*U. spelaeus*	1	Reguerillo	Tooth	-
T-2914	CB-CR4	*U. spelaeus*	1	Reguerillo	Tooth	-
T-4141	CB-CR5	*U. spelaeus*	1	Reguerillo	Tooth	-
T-4203	CB-CR6	*U. spelaeus*	1	Reguerillo	Tooth	-
T-4594	CB-CR7	*U. spelaeus*	1	Reguerillo	Tooth	-
TR-7000	CB-TR1	*U. spelaeus*	1	Troskaeta	Rib	-
TR-7001	CB-TR2	*U. spelaeus*	1	Troskaeta	Rib	-
TR-7002	CB-TR3	*U. spelaeus*	1	Troskaeta	Rib	-
TR-7003	CB-TR4	*U. spelaeus*	1	Troskaeta	Rib	-
TR-7004	CB-TR5	*U. spelaeus*	1	Troskaeta	Rib	-
TR-7005	CB-TR6	*U. spelaeus*	1	Troskaeta	Rib	-
TR-7006	CB-TR7	*U. spelaeus*	1	Troskaeta	Rib	-
TR-7007	CB-TR8	*U. spelaeus*	1	Troskaeta	Rib	-
AX-175-C1-62	CB-Am1	*U. spelaeus*	1	Amutxate	Rib	+
AX-344-C1-55	CB-Am2	*U. spelaeus*	1	Amutxate	Rib	+
AX-436-W3-15	CB-Am3	*U. spelaeus*	1	Amutxate	Rib	+
AX-1021-Y2-4	CB-Am4	*U. spelaeus*	1	Amutxate	Rib	+
AX-1034-X2-13	CB-Am5	*U. spelaeus*	1	Amutxate	Rib	+
AX-1069-X2-29	CB-Am6	*U. spelaeus*	1	Amutxate	Rib	+
AX-1264-Y2-33	CB-Am7	*U. spelaeus*	1	Amutxate	Rib	+
AX-4119-W-4-7	CB-Am8	*U. spelaeus*	1	Amutxate	Rib	+
AX-3640-U2-99	CB-Am9	*U. spelaeus*	1	Amutxate	Rib	+
AX-6809-S-3-16	CB-Am10	*U. spelaeus*	1	Amutxate	Rib	+
PUR-LU-75	BB-P1	*U. arctos*	1	Purruñal	Rib	+
LOME-1	LO1	*U. arctos*	1	Longo de Meu	Rib	+
SIPA-61	SP2	*U. arctos*	1	Pena Paleira	Tibia	+
SIPA-1	SP3	*U. arctos*	1	Pena Paleira	Rib	+
SIPA-166	SP4	*U. arctos*	1	Pena Paleira	Femur	+
SIPA-190	SP5	*U. arctos*	1	Pena Paleira	Rib	+
TA-176	BB-T1	*U. arctos*	1	Tarelo	Rib	+
Ta-Lu-C-60	BB-T2	*U. arctos*	1	Tarelo	Rib	-
SH5-98-528-082	S1	*U. arctos*	1	Somiedo	Rib	+
SH5-98-528-095	S2	*U. arctos*	1	Somiedo	Humerus	+
SH5-97-V29-1	S3	*U. arctos*	1	Somiedo	Rib	+
SH5-97-U29-59	S4	*U. arctos*	1	Somiedo	Rib	+
SH5-97-U28-7	S5	*U. arctos*	1	Somiedo	Rib	+
SH5-529-002	S6	*U. arctos*	1	Somiedo	Rib	+
CG-LL-050	PC1	*U. arctos*	1	Pozu la Cigacha	Ulna	+
CGLL-051	PC2	*U. arctos*	1	Pozu la Cigacha	Ulna	+
PUR-015	PV1	*U. arctos*	1	Pozu la Veiga'l Retuertu	Humerus	+
LCF-96-025	CF1	*U. arctos*	1	La Canal Fuerte	Rib	+
LCF-158	CF2	*U. arctos*	1	La Canal Fuerte	Rib	+
RT-001	RT1	*U. arctos*	1	Red de Tonello	Skull	+
CB-015	CB1	*U. arctos*	1	Cuetu Blanco	Rib	+
Uap	Uap	*U. arctos*	4	Winden, Austria	Petrous	N/A
PA1	PA1	*U. ingressus*	2	Arnautilor, Romania	5th Metacarpal	N/A
GS136	GS136	*U. ingressus*	3	Gamssulzen, Austria	Petrous	N/A

GS710	GS710	*U. ingressus*	3	Gamssulzen, Austria	Petrous	N/A
RK306	RK306	*U. s. eremus*	3	Ramesch, Austria	Petrous	N/A
SW1867	SW1867	*U. s. eremus*	3	Schwabenreith, Austria	Petrous	N/A
E-VD-1838	E-VD-1838	*U. spelaeus*	4	Eirós, Spain	Petrous	N/A
WK01	WK01	*U. s. eremus*	4	Windischkopf, Austria	Petrous	N/A

Table S3. Radiocarbon dates of the bear samples included in this study. ^{14}C dates dates were 2-sigma calibrated using OxCal 4.2 online, based on the IntCal-13 curve.

Sample ID	Taxon Ursus	Locality	Uncal. Age yBP	Uncertainty ±	Cal age yBP	Uncertainty ±	Remarks	Reference ^{14}C
E3	spelaeus	Eirós	30150	606	34310	1186		Pérez Rama et al. 2011
E16	spelaeus	Eirós	30210	160	34252	339		This study (Beta-395124)
E11	spelaeus	Eirós	30660	190	34583	389		This study (Beta-395123)
E-VD-1838	spelaeus	Eirós	30737	500	34806	931		This study (MAMS 23140)
E17	spelaeus	Eirós	30760	170	34674	365		This study (Beta-395125)
SP2091	spelaeus	Eirós	31390	865	35853	1961	FN390868 Eiros in Figure 3	Pérez Rama et al. 2011
L1	spelaeus	Liñares	46560	1050	-	-	out of cal range	This study (Beta - 343567)
L2	spelaeus	Liñares	>48000	-	-	-	Min age AND out of calibr range	This study (Beta - 345046)
L13	spelaeus	Liñares	49140	1590	-	-	out of cal range	This study (Beta - 345045)
L17	spelaeus	Liñares	>40000	-	-	-	Min age	Pérez Rama et al. 2011
C2	spelaeus	A Ceza	37830	390	42137	548		This study (Beta-391370)
C7	spelaeus	A Ceza	>43500	-	-	-	Min age	This study (Beta-391371)
C8	spelaeus	A Ceza	37490	370	41905	547		This study (Beta-391272)
C11	spelaeus	A Ceza	37240	330	41728	516		This study (Beta-391373)
A2	spelaeus	Arcoia	>40000	-	-	-	Min age	This study (Ua-41793)
US18	spelaeus	Chauvet France	31870	300	35734	643		Bon et al. 2008
SP2	arctos	Pena Paleira	7201	46	8053	107		García-Vázquez et al. 2015
SP3	arctos	Pena Paleira	6390	45	7340	83		García-Vázquez et al. 2015
SP4	arctos	Pena Paleira	6548	45	7452	115		García-Vázquez et al. 2015
SP5	arctos	Pena Paleira	6387	43	7340	83		García-Vázquez et al. 2015
S1	arctos	Somiedo	8720	40	9720	168		García-Vázquez et al. 2015
S2	arctos	Somiedo	8990	50	10085	159		García-Vázquez et al. 2015

S3	arctos	Somiedo	7100	40	7925	76		García-Vázquez et al. 2015
S4	arctos	Somiedo	4115	95	4635	215		García-Vázquez et al. 2015
S5	arctos	Somiedo	4160	30	4706	124		García-Vázquez et al. 2015
PC1	arctos	Pozu la Cigacha	6750	40	9260	170		García-Vázquez et al. 2015
PC2	arctos	Pozu la Cigacha	7340	40	8162	138		García-Vázquez et al. 2015
PV1	arctos	Pozu la Veiga’l Retuertu	9240	40	10393	130		García-Vázquez et al. 2015
CF1	arctos	La Canal Fuerte	9045	55	10158	216		García-Vázquez et al. 2015
CF2	arctos	La Canal Fuerte	8370	40	9388	94		García-Vázquez et al. 2015
Uap	arctos	Winden Austria	36680	500	41201	895		This study MAMS 23141
GS136	ingressus	Gamssulzen Austria	31026	500	35062	966		This study MAMS 23139
SP891	ingressus	Gamssulzen Austria	44160	+1400 -1190	-	-	may extend out of cal range	Krause et al. 2008
SP2106	ingressus	Geissenklösterle Germany	24210	100	28251	310		Stiller et al. 2010
SP2027	ingressus	Geissenklösterle Germany	27180	150	31142	215		Stiller et al. 2010
SP232	ingressus	Nixloch Austria	27230	140	31170	208		Hofreiter et al 2002
SP1626	ingressus	Pestera cu Oase Romania	46614	+1754-1638	-	-	out of cal range	Richards et al. 2008
SP234	ingressus	Potocka Zijalka Slovenia	26900	110	31000	190		Hofreiter et al 2002
PA1	ingressus	Romania	33600	400	37785	1083		This study (Poz-53626)
SP2064	ingressus	Serpievskaya Russia	44050	-	47754	576	no uncert.	Stiller et al. 2010

Table S4. Sequencing output and mapping statistics for the samples sequenced in this study. The samples with mtDNA coverage equal or higher than 70% where selected for further analysis and are highlighted in bold.

Sample DNA ID	Experiment	Total reads or read-pairs	Mapped reads	Unique mapped reads after FQ	% of non clonal Ursus mtDNA	coverage of mtDNA (%)	Depth of coverage
CB_E1	1	1011533	185891	138	0.01	7.97	0.58x
CB_E2	1	1882	1	0	0	0	0.0x
CB_E3	**1**	**1121003**	**227235**	**1265**	**0.11**	**90.68**	**5.64x**
CB_E4	1	1002857	344460	822	0.08	59.78	3.50x
CB_E5	1	764893	6360	222	0.02	11.55	0.85x
CB_E6	1	1234730	113481	885	0.07	59.14	3.66x
CB_E7	1	938322	5140	38	0.00	7.19	0.14x
CB_E8	1	770899	9820	58	0.00	1.6	0.22x
CB_E9	1	740915	26660	103	0.01	4.32	0.41x
CB_E10	1	500453	52089	133	0.02	37.84	0.63x
CB_E11	**1**	**807816**	**260068**	**1761**	**0.21**	**87.9**	**7.7x**
CB_E12	1	841125	1018	45	0.00	20.39	0.19x
CB_E13	1	1643495	956686	1529	0.09	69.45	5.84x
CB_E14	1	698113	65511	156	0.02	8.15	0.03x
CB_E15	1	657414	26458	179	0.02	8.59	0.67x
CB_E16	**1**	**6710813**	**3655720**	**7355**	**0.10**	**98.59**	**34.21x**
CB_E17	**1**	**2314996**	**740756**	**2838**	**0.12**	**90.02**	**12.51x**
CB_L1	**1**	**4211087**	**3090599**	**8044**	**0.19**	**98.63**	**37.43x**
CB_L2	**1**	**3267748**	**2408866**	**7698**	**0.23**	**98.69**	**36.57x**
CB_L3	1	785114	84247	419	0.05	31.01	1.78x
CB_L4	1	1075051	24993	100	0.00	27.43	0.45x
CB_L5	1	855839	200639	926	0.10	61.37	3.96x
CB_L6	**1**	**1834875**	**920942**	**3670**	**0.20**	**97.81**	**16.74x**
CB_L7	1	791508	24863	110	0.01	46.96	0.88x
CB_L8	1	1314167	492601	124	0.00	7.06	0.45x
CB_L9	1	721122	37017	768	0.10	58.16	1.21x
CB_L10	1	747636	42192	419	0.05	33.12	1.96x
CB_L11	1	806365	27454	213	0.02	11.06	0.85x
CB_L12	1	585966	19160	235	0.04	50.61	1.01x
CB_L13	**1**	**1426815**	**524451**	**3337**	**0.23**	**97.58**	**15.74x**
CB_L14	1	643125	64519	598	0.09	46.08	2.69x
CB_L15	**1**	**2160551**	**1057756**	**4313**	**0.19**	**97.48**	**19.07x**
CB_L16	1	1237653	16732	465	0.03	33.8	2.00x
CB_L17	**1**	**1226933**	**472125**	**4821**	**0.39**	**97.92**	**22.68x**
CB_L18	**1**	**1324976**	**405697**	**3072**	**0.23**	**97.89**	**14.35x**
CB_L19	**1**	**2212844**	**1212417**	**8801**	**0.39**	**98.37**	**41.03x**
CB_C1	**1**	**2635010**	**2313605**	**2465**	**0.09**	**97.68**	**11x**
CB_C2	**1**	**2240269**	**599811**	**2529**	**0.11**	**97.7**	**6.63x**
CB_C3	1	2422525	499169	1109	1.08	64.43	4.34x
CB_C4	**1**	**1659436**	**352289**	**1547**	**0.09**	**93.17**	**56.58x**
CB_C7	**1**	**6058983**	**3749984**	**11296**	**0.18**	**98.87**	**90.37x**
CB_C8	**1**	**14845195**	**11967199**	**19492**	**0.13**	**98.79**	**6.22x**
CB_C9	**1**	**1464923**	**518024**	**1366**	**0.09**	**96.82**	**43x**
CB_C10	1	1524762	311345	146	0.01	8.49	0.57x
CB_C11	**1**	**7325157**	**5163982**	**9329**	**0.12**	**98.64**	**10.94x**
CB_A1	**1**	**3617635**	**2266037**	**12071**	**0.33**	**98.42**	**57.7x**
CB_A2	**1**	**6073618**	**4628348**	**16445**	**0.27**	**98.36**	**74.38x**
CBAm1	1	830457	38478	372	0.04	21.08	1.42x
CBAm2	**1**	**1351825**	**730069**	**1544**	**0.11**	**97.26**	**7.44x**
CBAm3	1	700462	207227	707	0.10	55.88	0.03x
CBAm4	1	1076316	215624	828	0.07	59.86	3.82x

CBAm5	1	1051997	16346	17	0.00	4.35	0.08x
CBAm6	**1**	**8497862**	**5654970**	**9603**	**0.11**	**98.87**	**47.23x**
CBAm7	1	248477	92182	81	0.03	7.45	0.37x
CBAm8	**1**	**2492644**	**623870**	**3153**	**0.12**	**97.03**	**14.98x**
CBAm9	**1**	**1319093**	**480955**	**3390**	**0.25**	**94.28**	**14.29x**
CBAm10	**1**	**1360716**	**719698**	**4648**	**0.34**	**98.16**	**22.44x**
BB-P1	1	28423	432	169	0.59	4.8	0.67x
BB-LO1	1	37356	18346	245	0.65	13.9	1.18X
BB-SP2	**1**	**35864**	**16401**	**7090**	**17.76**	**97.04**	**24.76x**
BB-SP3	**1**	**26872**	**7457**	**3750**	**13.95**	**95.25**	**13.48x**
BB-SP4	**1**	**172763**	**118470**	**18805**	**10.88**	**98.47**	**79.67x**
BB-SP5	**1**	**65310**	**23875**	**6826**	**10.45**	**98.32**	**27.50x**
BB-T1	1	68527	24646	604	0.88	37.0	2.33x
BB-S1	**1**	**470193**	**209562**	**3721**	**0.79**	**98.87**	**17.32x**
BB-S2	**1**	**7157929**	**4268757**	**30250**	**0.42**	**99.31**	**154.62x**
BB-S3	**1**	**530574**	**338619**	**26340**	**4.96**	**98.96**	**133.03x**
BB-S4	**1**	**17423001**	**12839337**	**31541**	**0.18**	**99.4**	**161.19x**
BB-S5	**1**	**649553**	**449312**	**27270**	**4.19**	**99.28**	**137.08x**
BB-S6	**1**	**41956193**	**32335374**	**31075**	**0.07**	**99.38**	**169.43x**
BB-PC1	**1**	**756841**	**582521**	**30393**	**4.01**	**99.39**	**149.75x**
BB-PC2	**1**	**113039**	**72684**	**17953**	**15.88**	**99.02**	**89.77x**
BB-PV1	**1**	**463853**	**292744**	**23217**	**5.00**	**99.31**	**116.85x**
BB-CF1	**1**	**83245**	**10087**	**3786**	**4.54**	**98.50**	**18.56x**
BB-CF2	**1**	**39514**	**21850**	**8032**	**20.32**	**98.09**	**38.94x**
BB-RT1	1	65081	34830	194	0.29	8.6	0.89x
BB-CB1	1	33227	13604	487	1.46	22.15	1.88x
PA1	**2**	**6308503**	**5368705**	**6258**	**0.09**	**92.93**	**27.35x**
GS136	**3**	**358642648**	**58362**	**17061**	**5.6E-5**	**98.97**	**59.27x**
GS710	**3**	**53470789**	**9908**	**5968**	**1.4E-4**	**94.38**	**21.23x**
RK306	**3**	**58348187**	**2376**	**1795**	**3.7E-5**	**74.41**	**6.21x**
SW1867	**3**	**73311164**	**12044**	**8315**	**1.4E-4**	**98.40**	**21.98x**
E-VD-1838	**4**	**409117882**	**200086**	**31645**	**1.1E-4**	**98.59**	**86.31x**
WK01	**4**	**5272087**	**2139**	**2048**	**4.5E-4**	**82.23**	**4.88x**
Uap	**4**	**217267718**	**91037**	**27722**	**1.6E-4**	**98.91**	**87.36x**

Table S5. Optimal partitions and substitution models for the cave bear alignment, identified by PartitionFinder analysis

Subset	Best Model	Subset Partitions
1	K80+G	16s_pt2, COX1_CP1, COX2_CP1, COX3_CP1, CYTB_CP1, Cys, Met, ND1_CP1, ND3_CP1, ND4L_CP1, Val
2	HKY+G	ATP8_ATP6_CP2, COX1_CP3, COX2_CP3, D-loop, Leu1, Thr, Tyr
3	HKY+G*	ATP8_ATP6_CP1, COX1_CP2, COX2_CP2, COX3_CP2, CYTB_CP2, ND1_CP2, ND2_CP2, ND3_CP2, ND4L_CP2, ND4_CP2, ND5_CP2
4	HKY+G	COX3_CP3, CYTB_CP3, Gln, ND1_CP3, ND2_CP3, ND3_CP3, ND4L_CP3, ND4_CP3, ND5_CP3, ND6_CP1, ND6_CP3, Pro
5	HKY+G	12s, 16s_p1, ATP8_ATP6_CP3, Ala, Arg, Asn, Asp, Glu, Gly, His, Ile, Leu2, Lys, ND2_CP1, ND4_CP1, ND5_CP1, ND6_CP2, Phe, Ser1, Ser2, Trp

* PartitionFinder selected HKY+I for this partition, but the proportion of invariant sites parameter (+I) failed to converge and so a model with gamma distributed rate heterogeneity (+G) was used instead.

Table S6. Optimal partitions and substitution models for the brown bear alignment, identified by PartitionFinder analysis

Subset	Best Model	Subset Partitions
1	HKY	12s, 16s_p1, 16s_pt2, Arg, Asn, Asp, Cys, Gly, His, Ile, Leu1, Leu2, Lys, Phe, Ser1, Ser2, Trp, Val
2	HKY+G	ATP8_ATP6, Ala, COX2, CYTB, D-loop, Gln, Glu, Met, ND1, ND2, ND3, ND4, ND5, ND6, Pro
3	HKY	COX1, COX3, ND4L, Thr, Tyr

Supporting Table S7. Phylogenetic age estimates for samples without exact ^{14}C dates. Samples with additional sources of age information that allow validation of the phylogenetic estimates are indicated.

Sample	**Ref. / GB acc.**	**Taxon**	**Locality**	**Median phylogenetic estimate**	**Lower 95% HPD**	**Upper 95% HPD**	**Verification from other sources of age information**
A1	this study	*spelaeus*	Arcoia	42716.5104	36977.9267	49120.2185	
A2	this study	*spelaeus*	Arcoia	42759.1606	36712.6521	48694.9791	>40000 minimum age
AM10	this study	*spelaeus*	Amutxate	44757.4137	42082.2159	48460.6094	Amino acid racemisation 39000-48000, Torres et al. 2007
AM2	this study	*spelaeus*	Amutxate	43626.9087	40585.064	46910.2844	Amino acid racemisation 39000-48000, Torres et al. 2007
AM6	this study	*spelaeus*	Amutxate	44709.387	42150.494	48323.9369	Amino acid racemisation 39000-48000, Torres et al. 2007
AM8	this study	*spelaeus*	Amutxate	44205.5036	40764.6996	47900.6077	Amino acid racemisation 39000-48000, Torres et al. 2007
AM9	this study	*spelaeus*	Amutxate	44758.8483	41821.9968	48227.1955	Amino acid racemisation 39000-48000, Torres et al. 2007
C1	this study	*spelaeus*	A Ceza	41696.8207	39825.8625	43099.0424	
C4	this study	*spelaeus*	A Ceza	41661.5008	39531.7495	43144.6898	
C7	this study	*spelaeus*	A Ceza	42726.5987	36991.6049	48755.3714	>43500 minimum age
C9	this study	*spelaeus*	A Ceza	41886.6836	39344.1441	44278.6848	
FM177760	FM177760	*ingressus*	Gamssulzen Austria	36411.2193	30029.1474	41383.0405	may extend out of dating range
FN390843	FN390843	*ingressus*	Potocka Zijalka Slovenia	36453.5575	34545.1016	39495.7461	
FN390845	FN390845	*ingressus*	Gamssulzen Austria	35574.8502	33301.1793	37977.1715	
FN390846	FN390846	*ingressus*	Gamssulzen Austria	38989.3737	35099.773	43846.7136	
FN390847	FN390847	*s. eremus*	Herdengel Austria	66305.4285	50319.3657	84668.4928	
FN390848	FN390848	*ingressus*	Zoolithen Germany	49006.4945	37808.2973	60259.4916	
FN390849	FN390849	*spelaeus*	Zoolithen Germany	44259.9399	38752.5997	51260.4925	
FN390850	FN390850	*spelaeus*	Zoolithen Germany	55832.7796	46509.9313	67631.0643	
FN390851	FN390851	*spelaeus*	Zoolithen Germany	56896.1797	48210.1365	68972.2773	
FN390852	FN390852	*spelaeus*	Herrmans Germany	38086.9012	32781.327	44606.3701	
FN390853	FN390853	*ingressus*	Pestera cu Oase Romania	42001.736	35234.5775	49768.7309	may extend out of dating range
FN390854	FN390854	*ingressus*	Pestera cu Oase Romania	41638.4922	34911.3107	49671.5685	
FN390855	FN390855	*spelaeus*	Arcy Cure France	43719.6876	39231.9155	48083.0477	
FN390856	FN390856	*ingressus*	Divjie Babe Slovenia	46778.8716	38969.0312	55444.2282	
FN390857	FN390857	*ingressus*	Divjie Babe Slovenia	38083.1617	34715.1044	41992.3577	
FN390858	FN390858	*ingressus*	Divjie Babe Slovenia	39426.5785	36238.9555	42957.2007	

FN390860	FN390860	*ingressus*	Bolshoi Glukhoi Russia	48335.8968	45894.4291	51163.1776	
FN390862	FN390862	*ingressus*	Medvezhiya Russia	47697.9878	44455.3773	50396.908	
FN390865	FN390865	*spelaeus*	Liñares	40543.3529	35396.6723	47250.1158	
FN390866	FN390866	*spelaeus*	A Ceza	39987.0777	36787.4503	42666.8316	
FN390867	FN390867	*spelaeus*	A Ceza	41295.7008	38523.897	44007.8205	
FN390870	FN390870	*ingressus*	Medvedia Jaskyna Slovakia	40447.1646	34555.8884	48859.134	
FN390871	FN390871	*spelaeus*	Dours France	75310.0697	58719.9315	94971.2477	
FN390872	FN390872	*s. eremus*	Schneiberhoehle Germany	77898.766	61623.2228	98317.0893	
GS710	this study	*ingressus*	Gamssulzen Austria	35176.1799	32200.8146	37850.9277	
L1	this study	*spelaeus*	Liñares	42745.3388	36927.0432	48749.2155	out of calibration range
L13	this study	*spelaeus*	Liñares	42566.7529	36873.5927	48716.7377	out of calibration range
L15	this study	*spelaeus*	Liñares	42661.3652	36788.4411	48527.3635	
L17	this study	*spelaeus*	Liñares	42795.0825	36871.9366	48722.2961	>40000 minimum age
L18	this study	*spelaeus*	Liñares	42213.8327	36602.7642	48517.4168	
L19	this study	*spelaeus*	Liñares	42453.8535	36240.0254	49016.6254	
L2	this study	*spelaeus*	Liñares	42711.8201	36894.4519	48726.7191	>48000 minimum age and out of calibration range
L6	this study	*spelaeus*	Liñares	42622.67	36773.2518	48577.8352	
RK306	this study	*s. eremus*	Ramesch Austria	71999.2234	53302.031	92678.0381	
SW1867	this study	*s. eremus*	Schwabenreith Austria	69228.1823	54310.5047	89624.5317	
WK01	this study	*s. eremus*	Windischkopf Austria	71991.6346	54640.0508	91860.1299	
S6	this study	*arctos*	Somiedo	7961.5195	5348.5502	9775.6109	

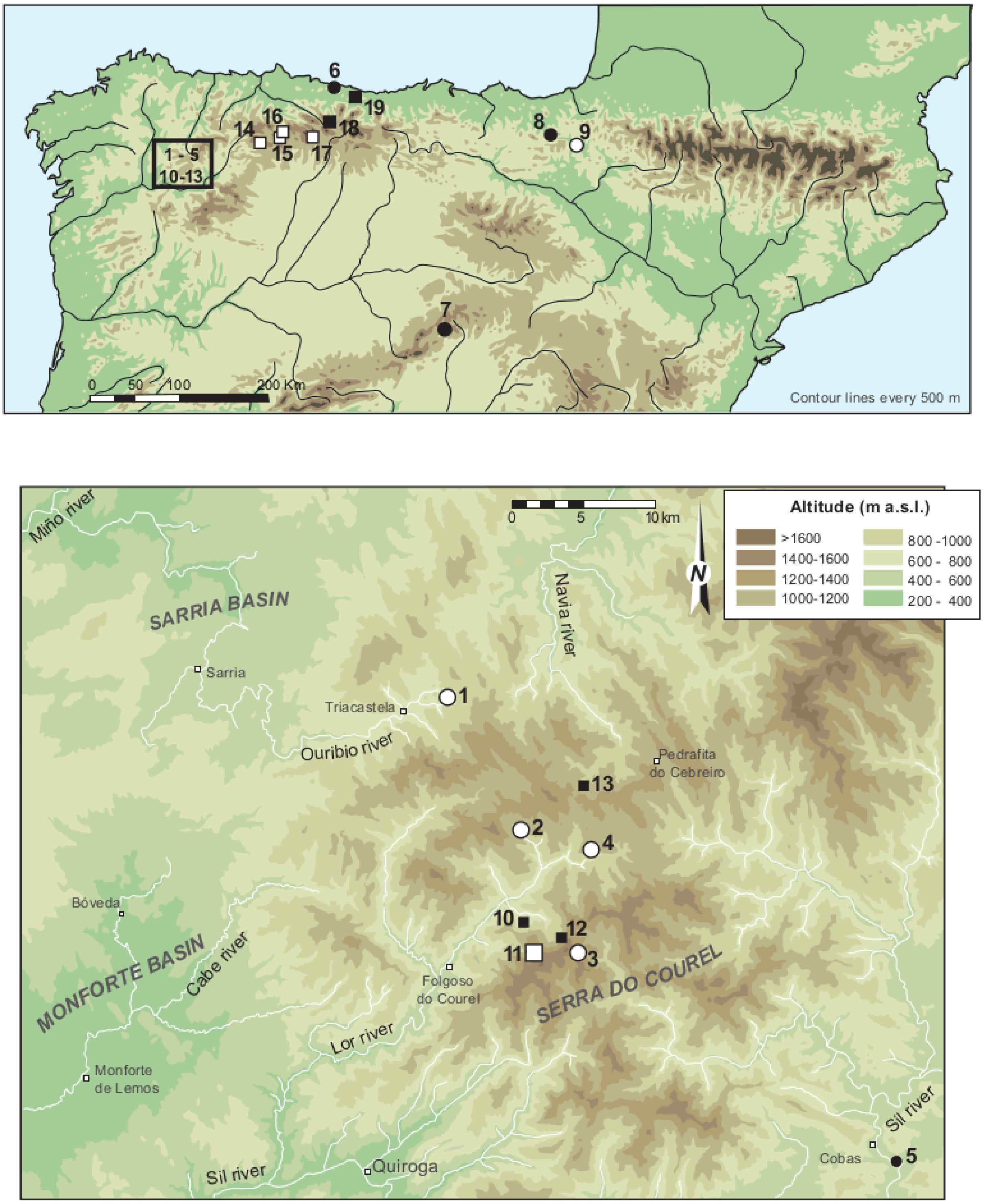

Figure S1. Map showing the location of the studied sites in Spain. Caves containing cave bear remains are represented by circles, while the caves containing brown bear remains are represented by squares. Filled black markers indicate sites that failed initial PCR screening for endogenous DNA in Experiment 1.

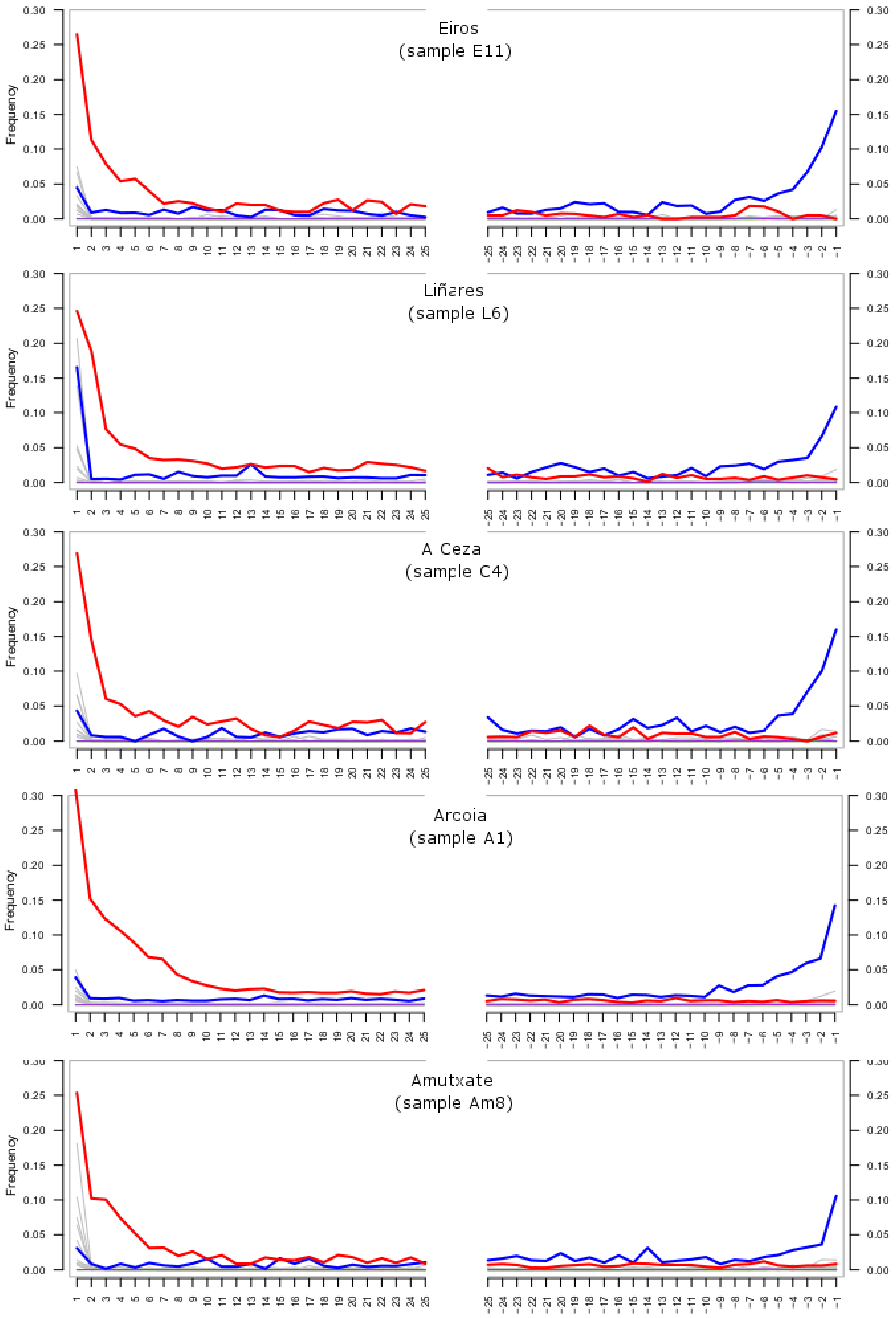

Figure S2. Deamination patterns at the ends of cave bear sequencing reads. One sample is shown as a representative example from each cave. Shown on the left is the C to T deamination rate at the 5' end (red line), and on the right the G to A rate at the 3' end (blue line). The lower G to A deamination rate observed at the 3' end compared to the C to T deamination at the 5' end is likely the results of fragments longer than 100bp in the library, for which the 3' end has not been sequenced using 100bp SE sequencing.

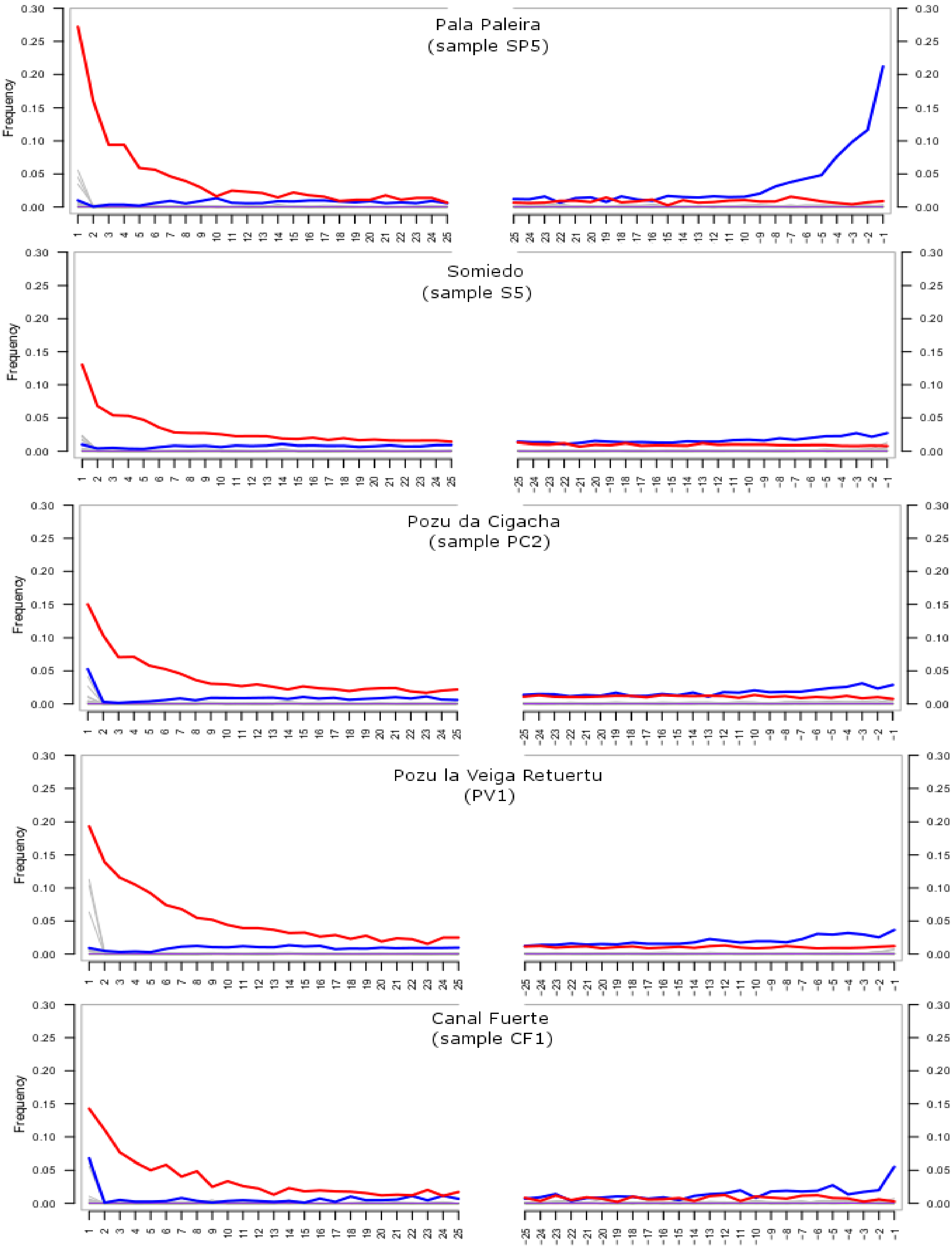

Figure S3. Deamination patterns at the ends of brown bear sequencing reads bears. One sample is shown as a representative example from each cave. Shown on the left is the C to T deamination rate at the 5’ end (red line), and on the right the G to A rate at the 3’ end (blue line).

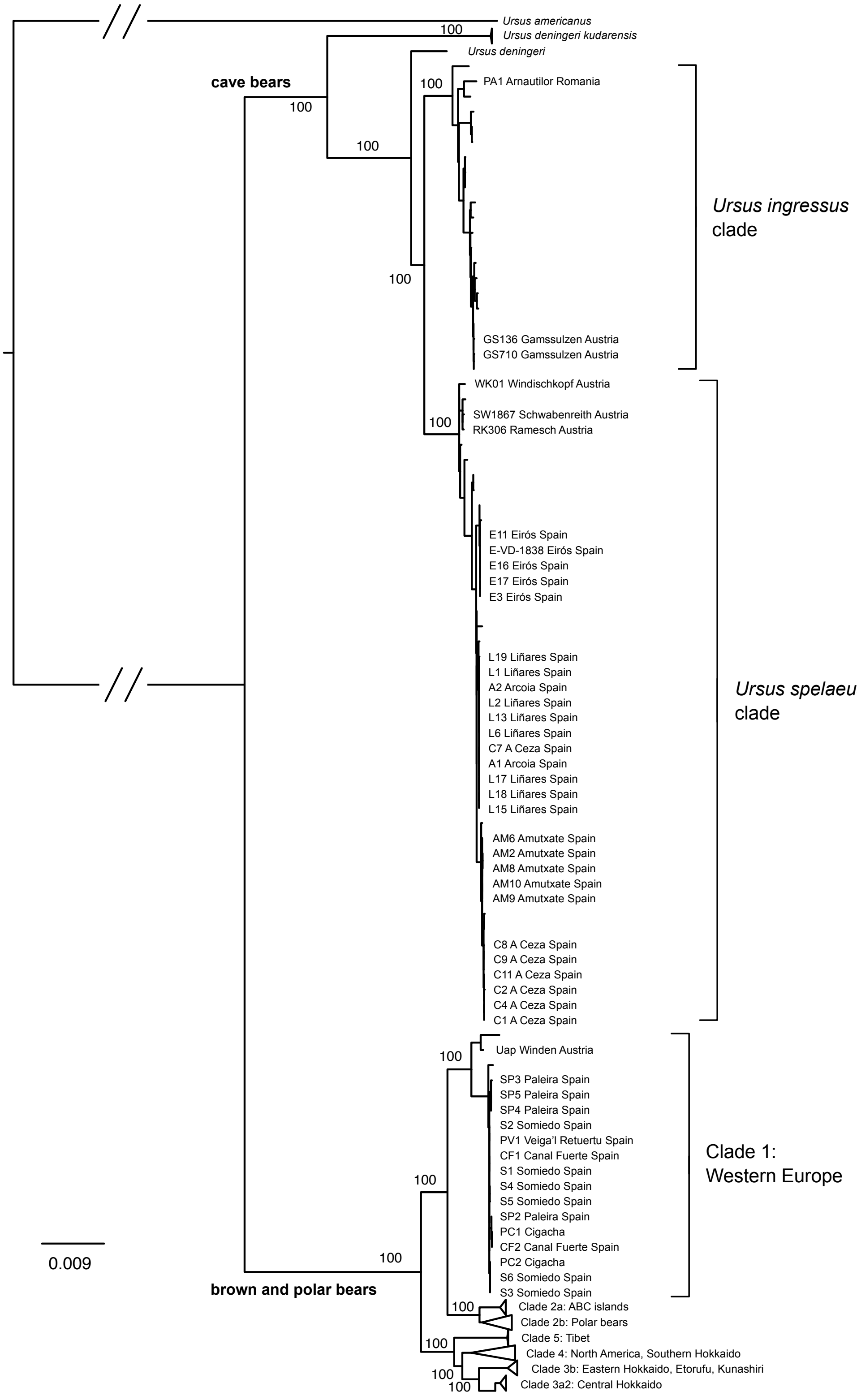

Figure S4. Maximum-likelihood phylogeny of cave bear, brown bear and polar bear mitochondria, rooted with *U. americanus*. Branch labels show bootstrap support for major clades. Scale bar shows substitutions per site. Note that polar bear mitochondria are nested within brown bear mitochondrial diversity. Labelled tips indicate novel sequences generated in this study. Clades that do not contain novel sequences are collapsed for simplicity. Branches extending from the tree root are reduced for better ingroup visualisation (indicated by "//"). Clade names for brown bears and polar bears follow the scheme of Hirata *et al.* (2013).

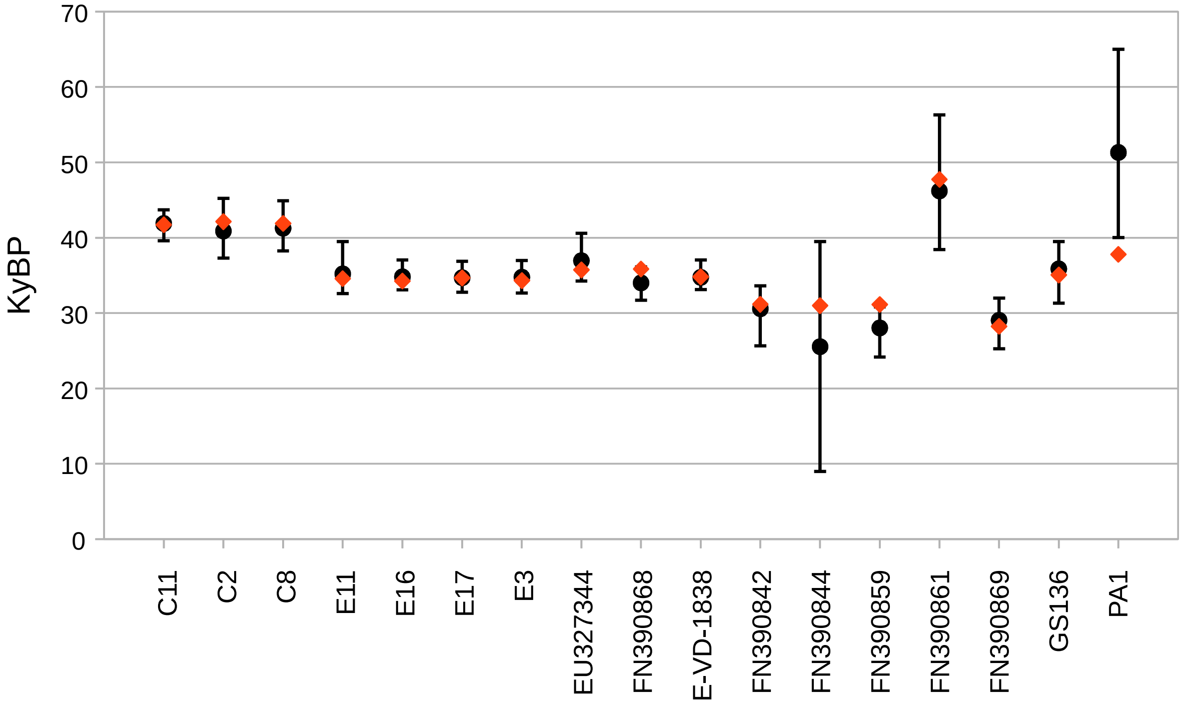

Figure S5. Results of crossvalidation tests for ^{14}C dated cave bear samples. Red points show ^{14}C ages. Black points show median phylogenetic estimates for that sample based on all other dated samples. Error bars show the 95% HPDs of the phylogenetic estimate. In general, the estimated ages closely match the actual radio-carbon ages, validating the accuracy of the procedure. In only one case, sample PA1, the 95% HPDs do not encompass the actual radio-carbon age.

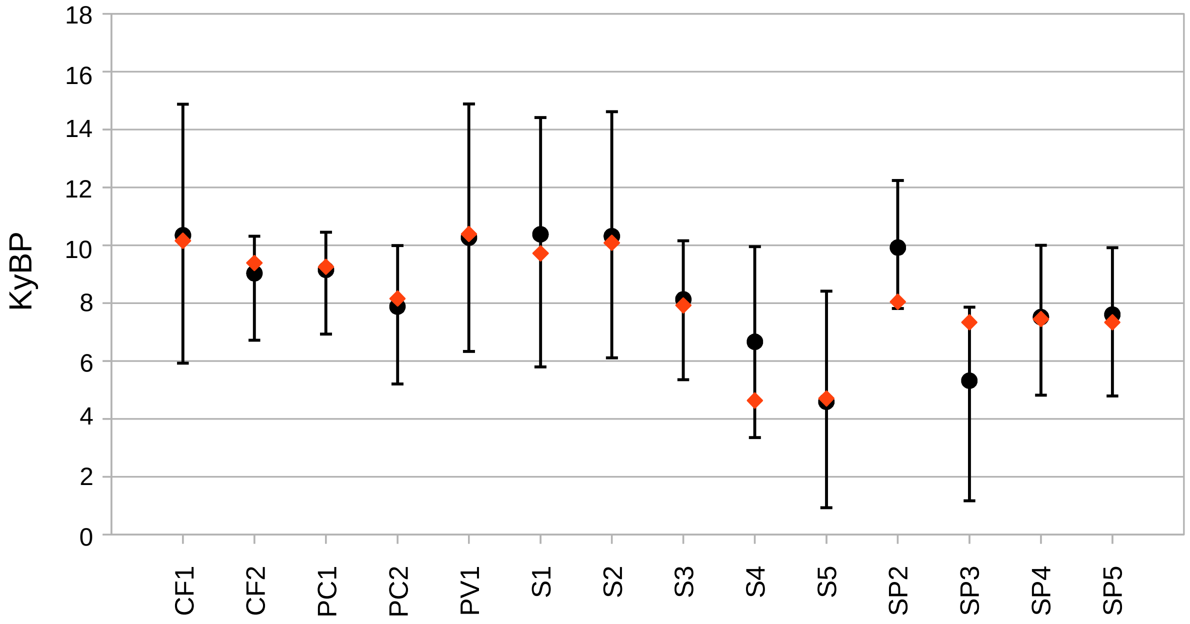

Figure S6. Results of crossvalidation tests for ^{14}C dated brown bear samples. Red points show ^{14}C ages. Black points show median phylogenetic estimates for that sample based on all other dated samples. Error bars show the 95% HPDs of the phylogenetic estimate. In general, the estimated ages closely match the actual radio-carbon ages, validating the accuracy of the procedure. Data from a Late Pleistocene Austrian brown bear (Uap) was used for age estimation, but the ability to estimate the age of this sample phylogenetically from the other brown bear ages was not considered for the purpose of cross validation tests. The ages of all other dated brown bears are so far removed from the Late Pleistocene bear that this analytical method is unable to generate any meaningful estimate.

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